An Integrated/Modular Sample Handling and Biotechnology Life Detection Instrument for Solar System Exploration

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James Schmitt	Montana State University	Co-I
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Pascal Lee	SETI Institute	Co-I
Charlie Cockell	SETI Institute	Co-I
Wendell Mendell	NASA JSC	Collaborator
M. Wuttke	Department of Natural History, Mainz	Collaborator
Ken Nealson	USC	Collaborator

Philosophy

To define a non-rover mobile platform containing remote sample preparation capabilities including; sample acquisition, drilling or sub coring and secondary sample processing that can be used by any instrument for testing in any Mars relevant environments.

To make this system light, flexible, easy to integrate to and available to any instrument team for use in their testing.

To show this by using existing instrumentation combined with instruments under development in several environments.

To test in field and laboratory off the shelf instrumentation to measure viable and fossil organic biosignatures in all environments to verify instruments under development. Includes detection sensitivity, performance in the field and contamination issues.

Science Objectives

S1	Define suitable probes (e.g., DNA, aptamers, antibodies) for several terrestrial field sites through assay in the laboratory of returned samples from these sites.
S2	Identify optimal techniques for *in-situ* extraction of important biomarkers located in rocks, soil, and liquid samples.
S3	Test DNA/protein micro-array technology, PCR, fluorescence, and enzyme based life detection instruments in the above environments.
S4	Integrate these with the life detection instruments including SOLID (DNA/ protein chip technology), PCR, LAL enzyme assay, ATP fluorescence, microscopy and fluorometric analysis.
S5	Test regimes to ensure correct mineral and pH buffering for each technique mentioned above.
S6	Study *in-situ* terrestrial microbial populations associated with solar system relevant environments
S7	Define the detection sensitivity of a suite of biotechnology instruments in the field.

Technology Objectives

T1	Develop an integrated robotic biotechnology platform to acquire samples and detect life in Astrobiology relevant environments. Use of Ultrasonic corer to process drill cores.
T2	Develop requirements and a design for an autonomous sample acquisition and handling system capable of obtaining and processing soil, rock, and liquid samples from a variety of relevant environments.
T3	Integrate custom and off-the-shelf biotechnology instruments with a custom microfluidics system to produce a compact suite of instruments.
T4	Refine the instrument suite design based upon the results of field tests.

Field Campaign Objectives

F1	Integrate laboratory testing with the first phase of field testing in a “safe”, “close-to-services” environment.
F2	Determine the detection sensitivity of a suite of biotechnology instruments in relevant environments. Coordinate logistics including shipping expensive technology, testing and assembling a field lab, and using video cons and interactive web page design for debriefing and dissemination of “lessons learned.”
F3	Test Instrumental capabilities in the field including a) Communications technology; b) Robotic aspects; c) Power requirements and capabilities; d) Base plate flexibility and functionality; e) Computer integration; and f) System integration in the field; g) Test biotechnology instruments.
F4	Test Environmental effects in the field to determine whether the instrument can withstand and function up to specifications in different conditions of a) Lanscape form; b) Substrate form (soil vs. rock); c) Ambient Temperature; d) Relative Humidity (Aqueous to Dry); e) Salinity; and f) Weather, e.g., Wind, Dust, or Precipitation.

INITIAL PROPOSED FIELD SITES

v Home Station, NASA JSC
v Rio Tinto, Spain
v Hot Springs (Yellowstone Caldera)
v Deep Sea Hydrothermal Systems (without described platform)
v Enspel Formation, Enspel, Westerwald, Germany (Fossil Life Relevant)
v Australian Desert Soils and Precambrian Rocks (Marble Bar and the Apex Chert)
v Haughton Crater, Devon Island, Canada

Each field location had a different PI and organizing scientists and was designed to test different Mars relevant scenarios over a three year period.

Technology

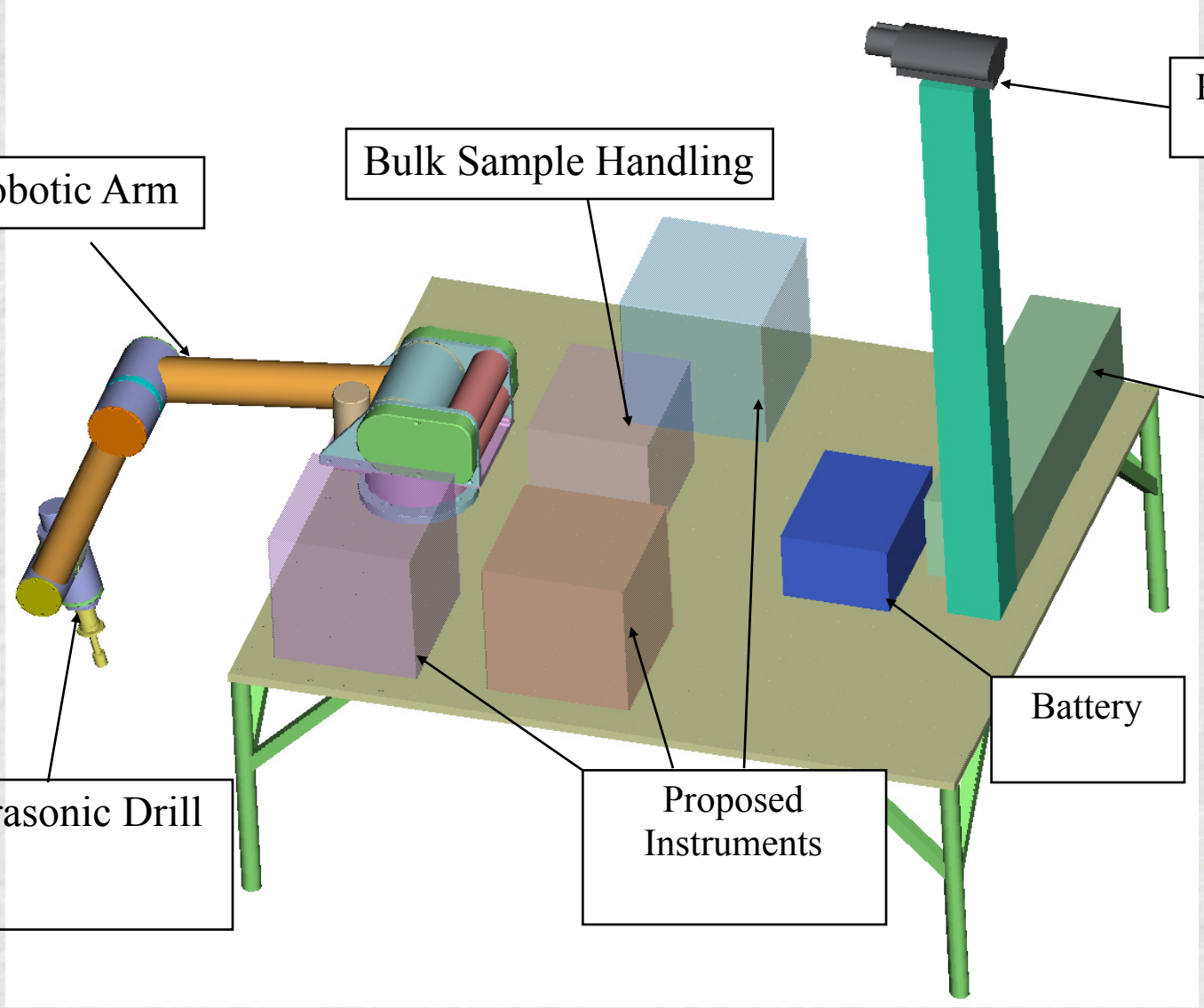
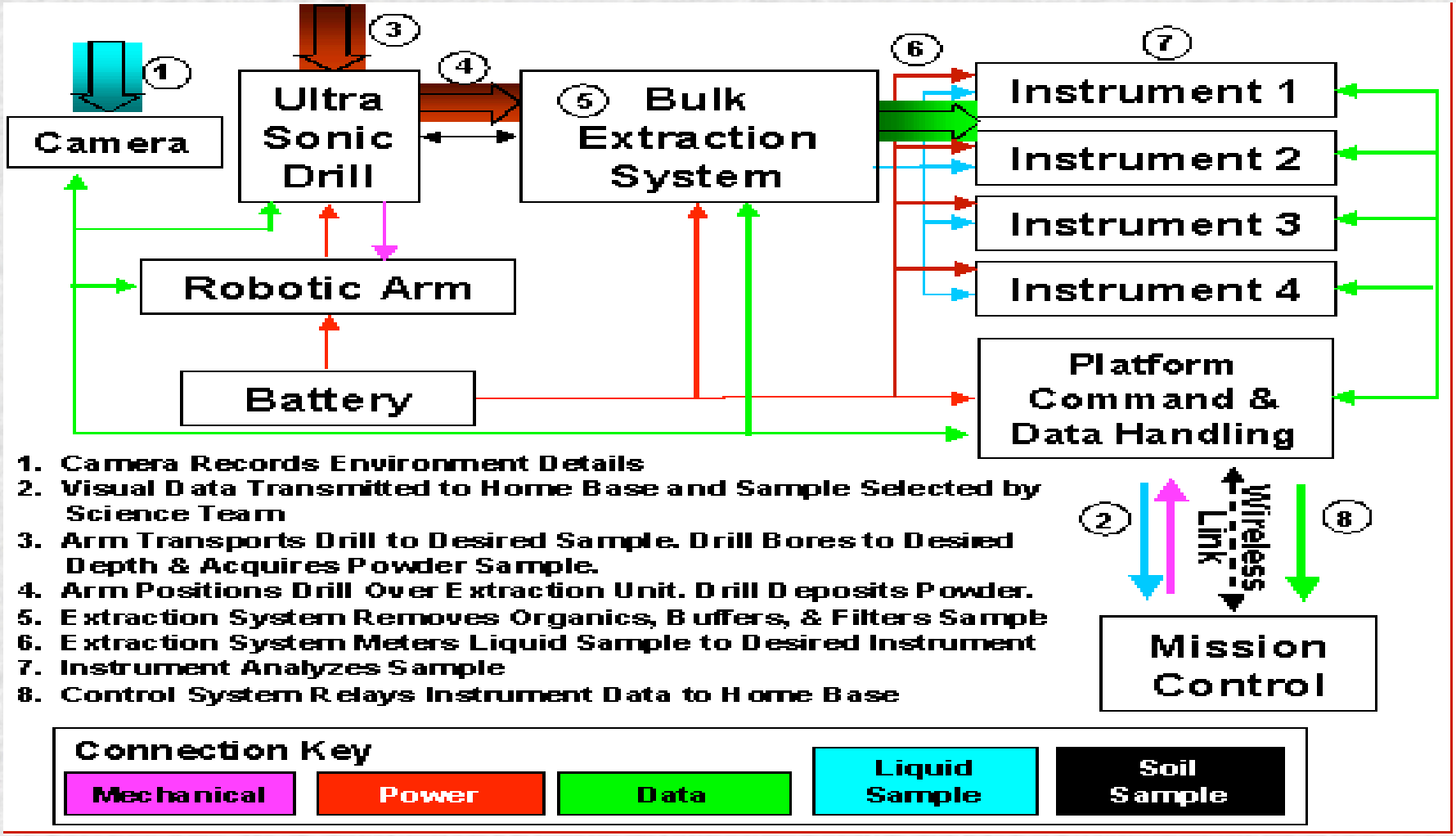
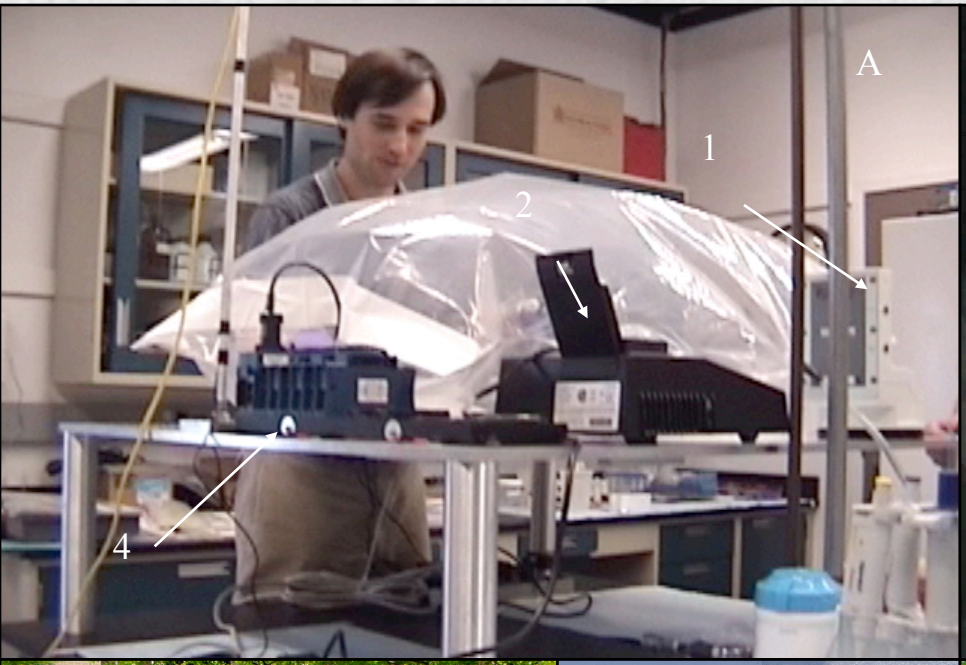

Remote Camera
Robotic Arm
Bulk Sample Handling
Data communications and control system
Battery
Ultrasonic Drill
Proposed Instruments

Table 2	Mass	Dimensions	Power (W)
SimRP Platform	41.4kg	104.2cm x 34.3cm x 94.0cm	Nil
Robotic Arm	7.5 Kg	Reach 101 cm	25-75
Drill	> 1 Kg	25cm inches drill depth of 12-13 cm	5-7
	Voltage (VDC)	Current (mA)	Power (W)
Panasonic Camera	12	780	9.36
NI Compact FieldPoint System	20	800	16

Platform



Where possible the platform was designed to use off the shelf components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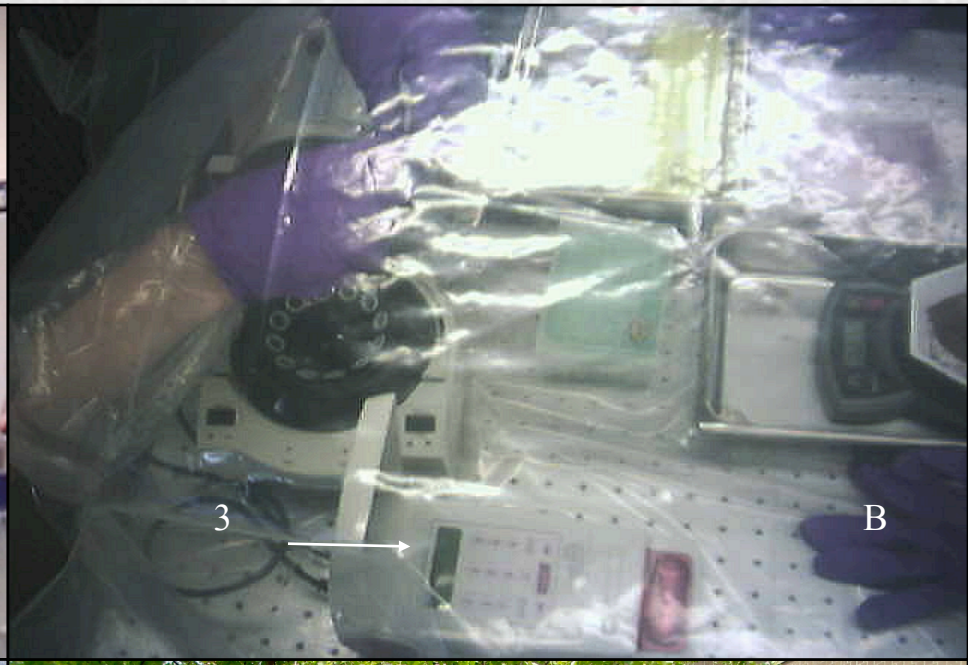
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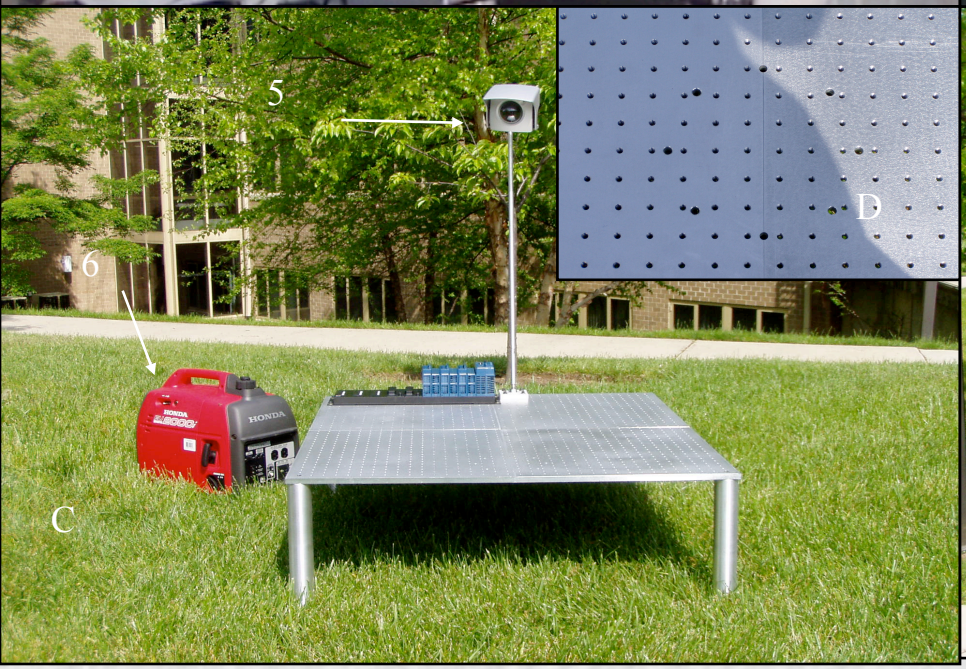
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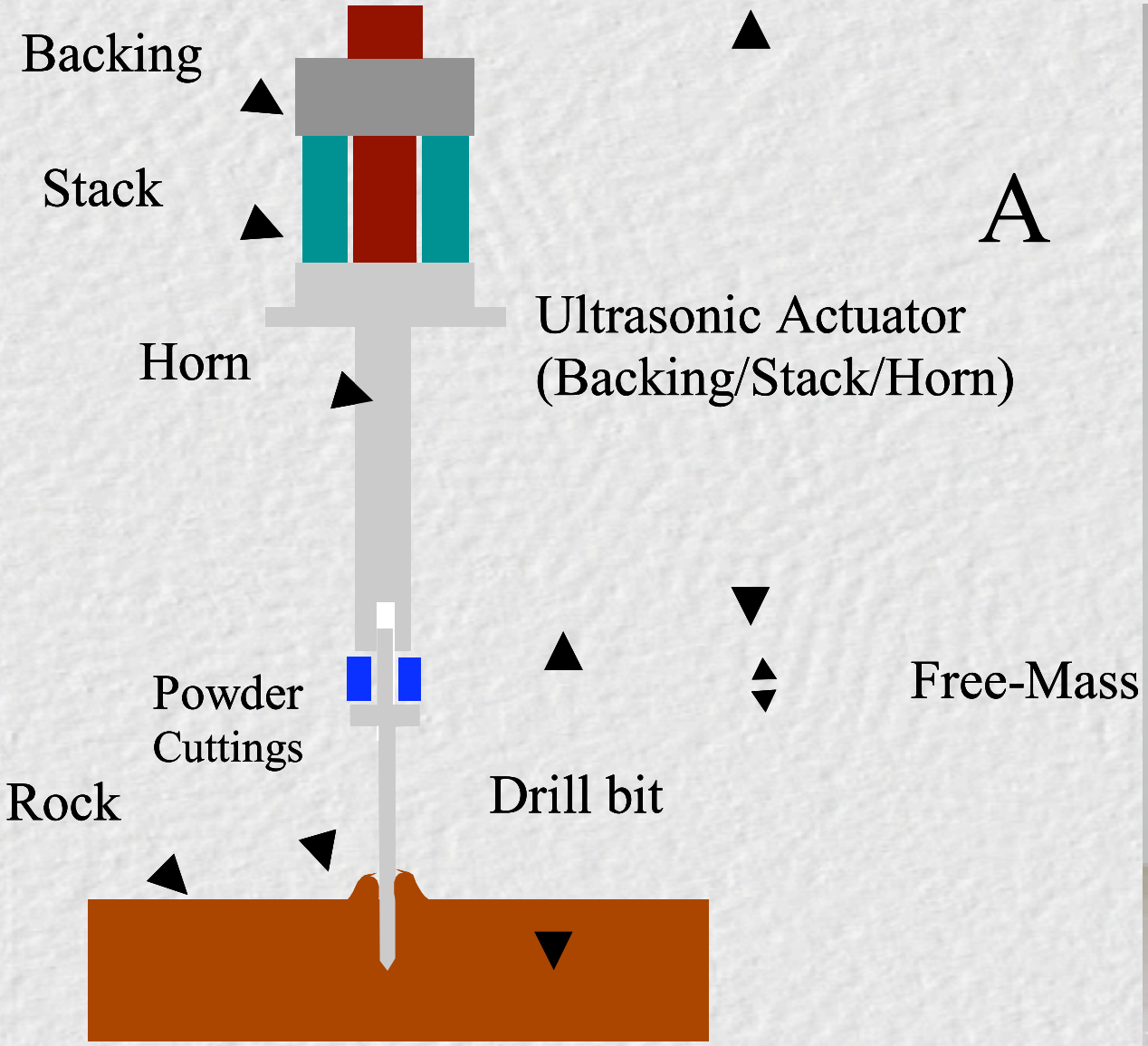
HONDA
EU2000

D

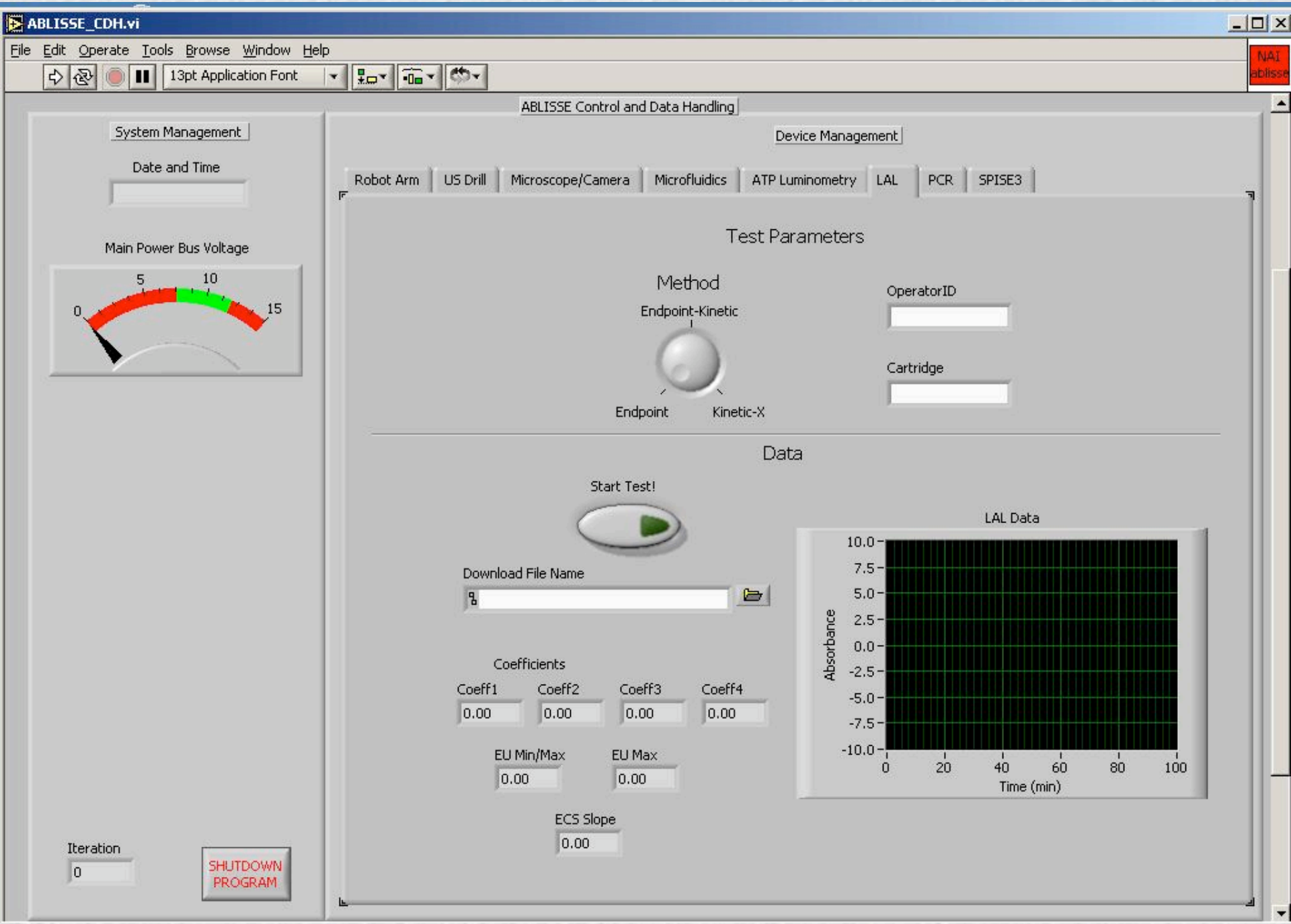

E


OCEANEERING
TERABOT


Backing
Stack
Horn
Ultrasonic Actuator
(Backing/Stack/Horn)
A
Free-Mass
Powder
Cuttings
Rock
Drill bit


B

- Direct connectivity to sensors and actuators
- 8 and 16-channel modules; individually configurable channels
- Hot-swappable and autoconfigurable
- Programmable power-up states
- -40 to 70 °C operating range, 50G shock 5G vibration
- 8 Analog and Digital I/O Modules for Compact FieldPoint
- LabVIEW Real-Time embedded controllers for control, measurement, and signal processing
- Operate as stand-alone embedded real-time controllers or PC-based distributed I/O Ethernet interface (connection via satellite phone).
- Embedded Web and file servers with remote panel user interface with real time updates
- Nonvolatile memory for embedded program storage and data logging
- Onboard DRAM memory for embedded program operation
- 2 RS-232 and 1 RS-485 serial ports for connection to peripherals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Iteration
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SHUTDOWN PROGRAM
Device Management
Robot Arm
US Drill
Microscope/Camera
Microfluidics
ATP Luminometry
LAL
PCR
SPISE3
Test Parameters
Method
Endpoint-Kinetic
Endpoint
Kinetic-X
OperatorID
Cartridge
Data
Start Test!
Download File Name
Coefficients
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Coeff2
Coeff3
Coeff4
0.00
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EU Max
ECS Slope
LAL Data
Absorbance
10.0
7.5
5.0
2.5
0.0
-2.5
-5.0
-7.5
-10.0
0
20
40
60
80
100
Time (min)


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Comm Link
Transmit in Progress
Iteration
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SHUTDOWN PROGRAM
Device Management
Robot Arm
US Drill
Microscope/Camera
Microfluidics
ATP Luminometry
LAL
PCR
SPISE3
Health and Status
Drill Temperature (C)
300
250
200
150
100
50
0
Drill Head Pressure (psi)
0
10
20
30
40
50
60
70
80
90
100
Drill Depth/Rate
10.0
8.0
6.0
4.0
2.0
0.0
0
100
Time (s)
Drill Depth (mm)
Drill Rate (mm/s)
Overheat Warning
Overpressure Warning
Calculated Drill Rate (mm/s)
0.00
Drill Limit
Command
Drill Motor
On
Off
Drill Depth Limit (mm)
0.00
Drill SHUTDOWN!
Drill SHUTDOWN Override

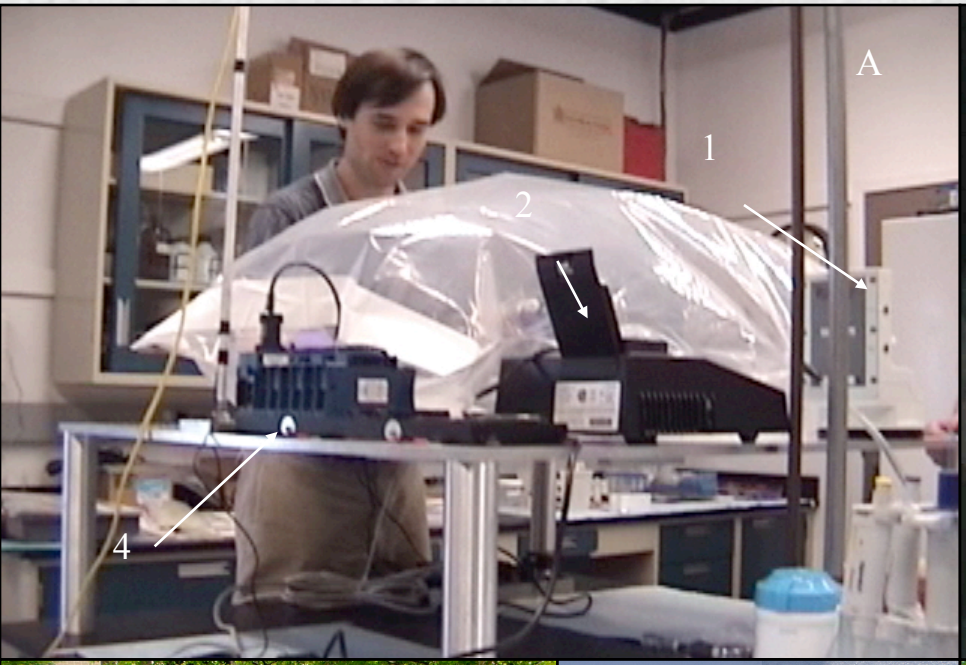
Bulk Sample Handling and Delivery System

Provide Liquid sample handling.
Initially provided by CAB – SOLID instrument or MASSE instrument

Neither instrument are currently ready for field deployment and therefore a separate sample handling has been designed and is currently being fabricated.

· Wetting and mixing of the initial soil sample
· pH Buffering and Metered Reagent Delivery
· Heated Incubation (if needed)
· Macro-filtration to remove particulates
· Micro-filtration and pre-concentration of sample.
• Metered microfluidic delivery of sample to each analysis instrument

• Initially until completed a manual fluid interface would be used.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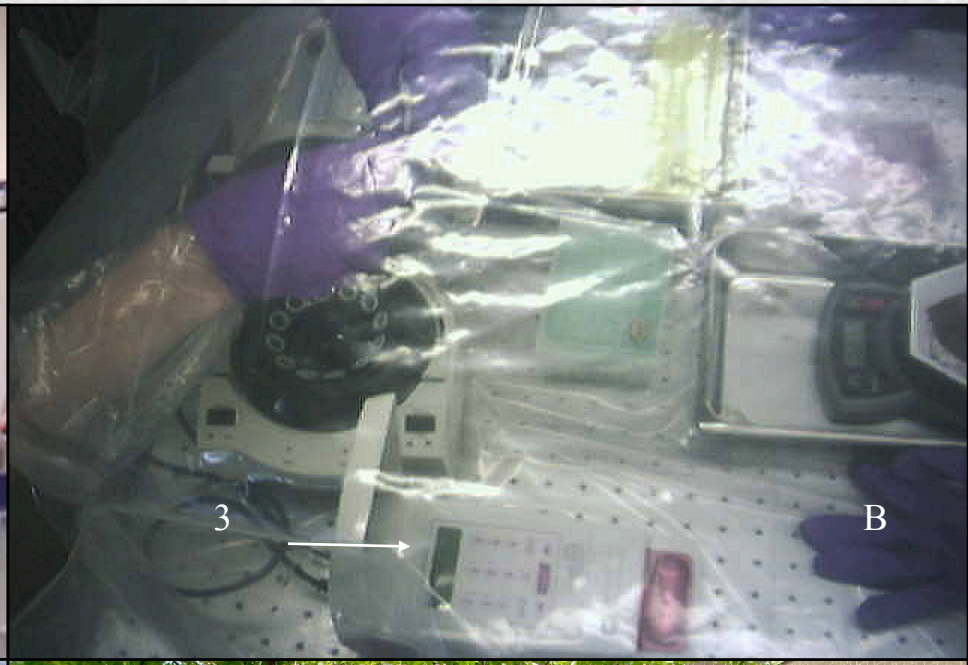
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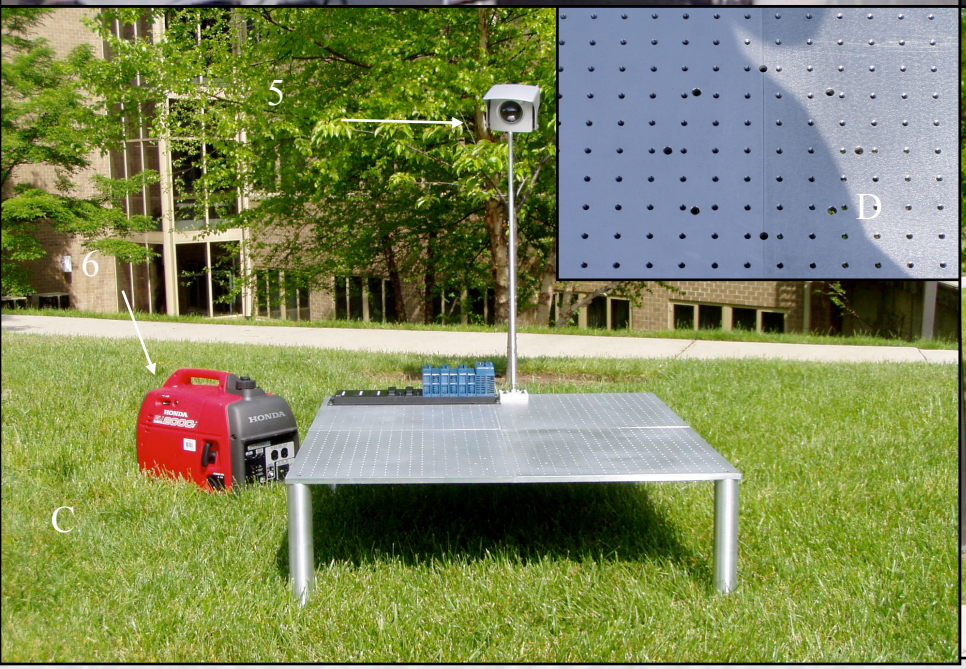
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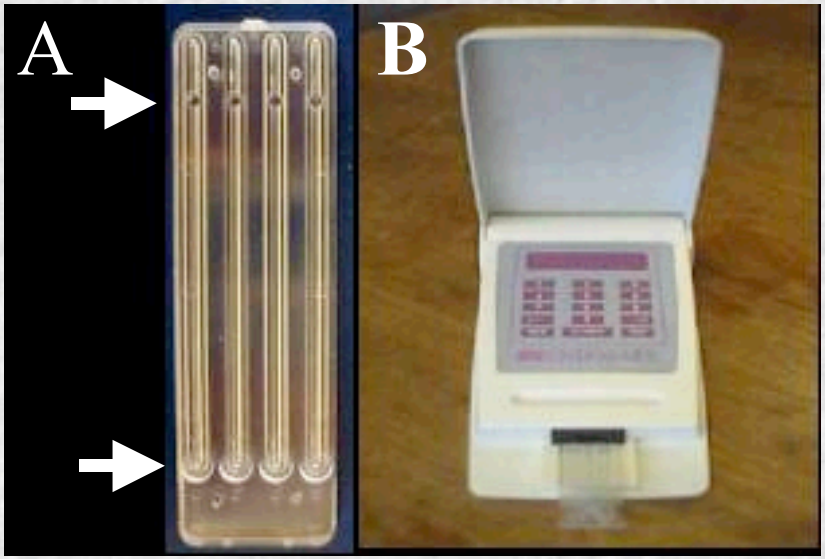
INSTRUMENTS

The instrument suite can be used both as a primary biological detection suite or to verify the measurements made on other instruments

Top arrow shows Optical Chamber where the reaction is measured



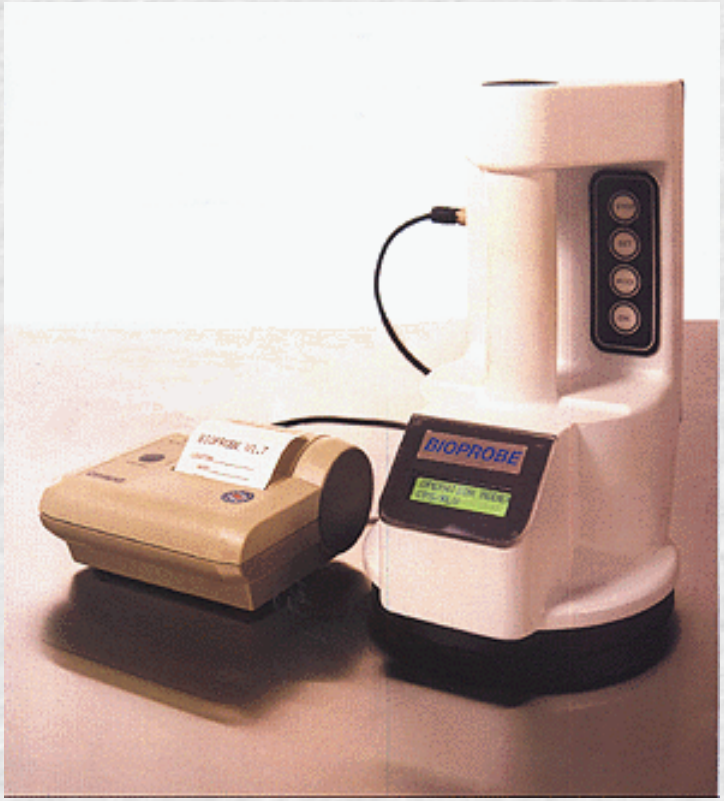
Bottom arrow shows 25 μL Sample Chamber

Limulus Aemebocyte Lysate (LAL)

Charles River Endosafe unit, N Wainwright MBL.

Detects Endotoxin (LPS) and is being used in Planetary Protection projects including having swabbed the MER rovers to detect bio-load of Gram Negative bacteria. Being developed for space flight.

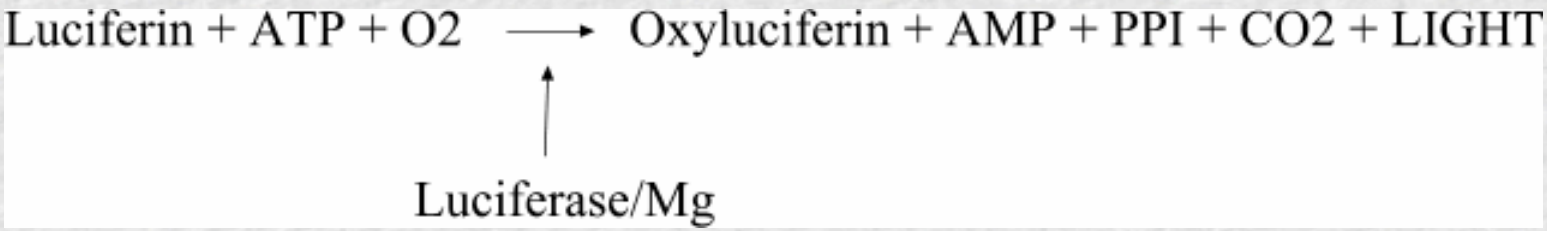
Detection Sensitivity – 1 Cell (if you can get it into the machine)
Extraction



ATP luminometer

Reads amount of ATP within seconds. Records metabolic activity in solid, liquid and swabbed samples. Being developed for space flight.

Detection sensitivity $10^2 – 10^3$ cells.



Mobile PCR

Complete mobile molecular laboratory. Enables extraction, PCR, Gel loading, Gel imaging within a single case.

PCR is as sensitive as extraction method.

Protocols have been optimized to ensure no contamination whilst in the field.

Several extraction methods have been studies including MoBio, (10^4 – 10^6 cells), DNA pure (10^6) and FTA paper (10^2 cells in solution).

DG2 Digital Microscope Scalar scopes.

Lenses contain light source and are made to be in focus upon contact with the surface of interest.

Includes, x30 – x 1000 lenses. UV, bore hole, endoscope attachments.

Others in the pipe line or in the proposal-

SPISE3 – Pan Conrad.

Hand held Capillary Electrophoresis

Hand Held PCR instruments

RT-PCR Instruments

Portable Raman spectrometer

Deployment

LAL – Planetary protection, Chesapeake bay, CIW, Svalbard (AMASE), rio Tinto.

ATP – Belize, Enspel, Chesapeake bay, Svalbard, rioTinto.

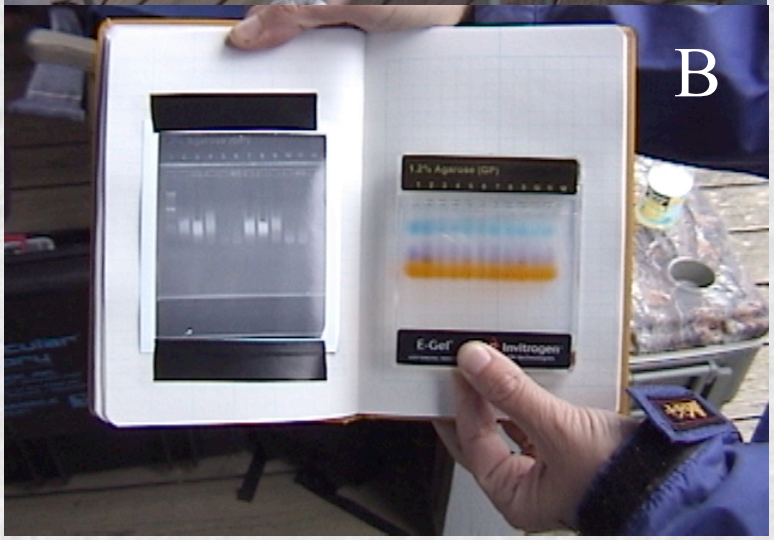
PCR – Chesapeake bay, Svalbard, rio Tinto, MSFC

Microscopy - Belize, Enspel, Chesapeake bay, Svalbard, rioTinto, Taiwan.

To come – supporting P Conrad ASTEP, Taiwan (deep drilling), Italy (drilling volcanoes), Svalbard.

Chesapeake Bay

Test contamination and field trip protocols in a near service environment.



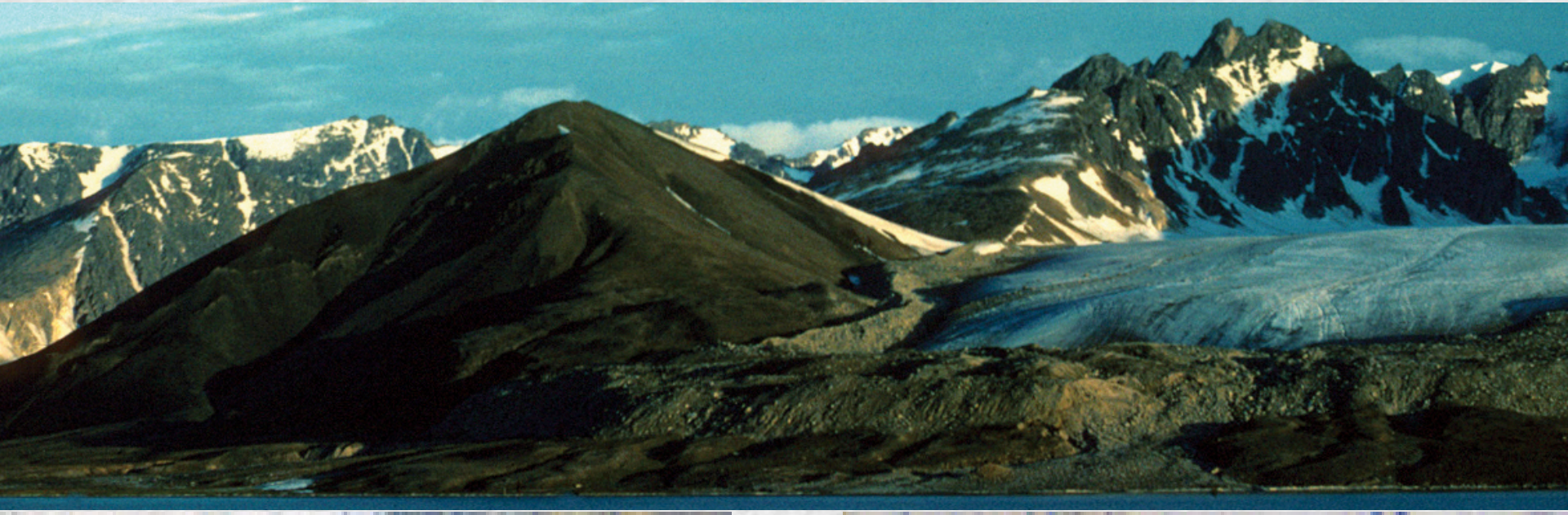
PCR – 16S rDNA, Archaea specific, Ammonia Oxidase and Nitrate reductase.

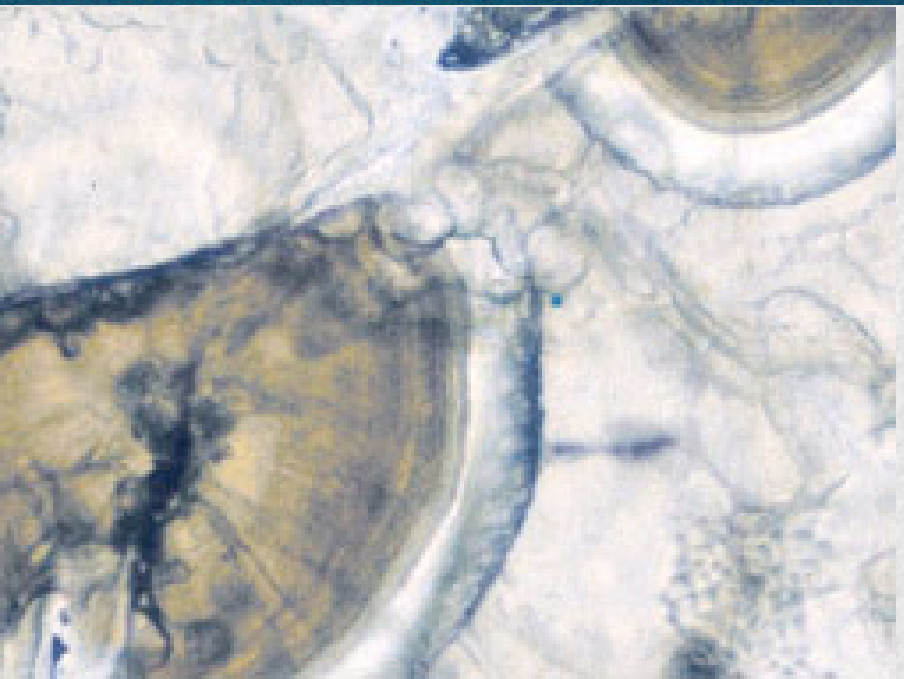
All negatives were negative, including extraction blanks.

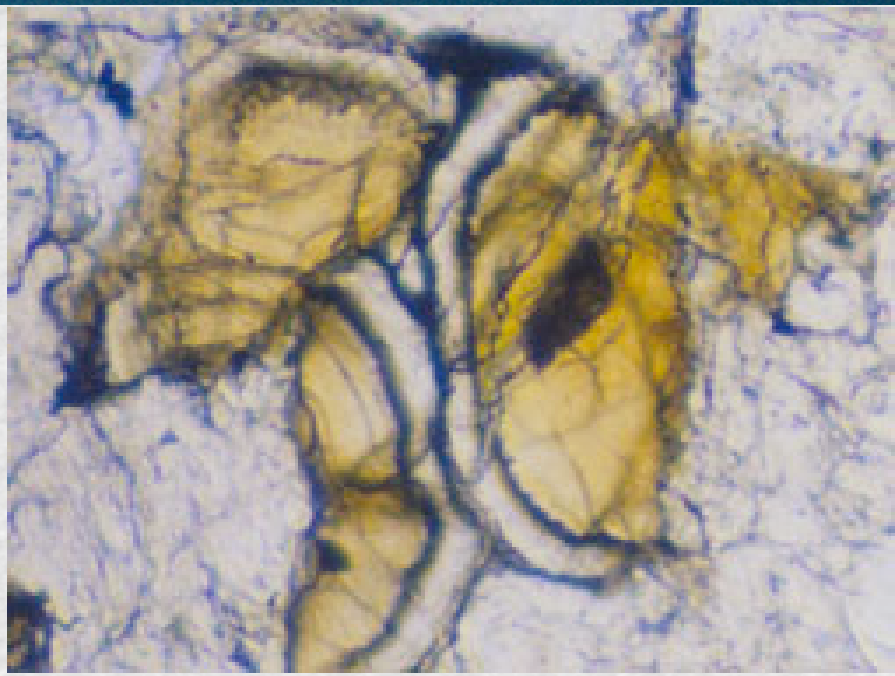
LAL and ATP worked well, results showed 10^6 – 10^8 cells within the ore taken for PCR.

Arctic Mars Analogue Svalbard Expedition (AMASE)

Why Svalbard ?







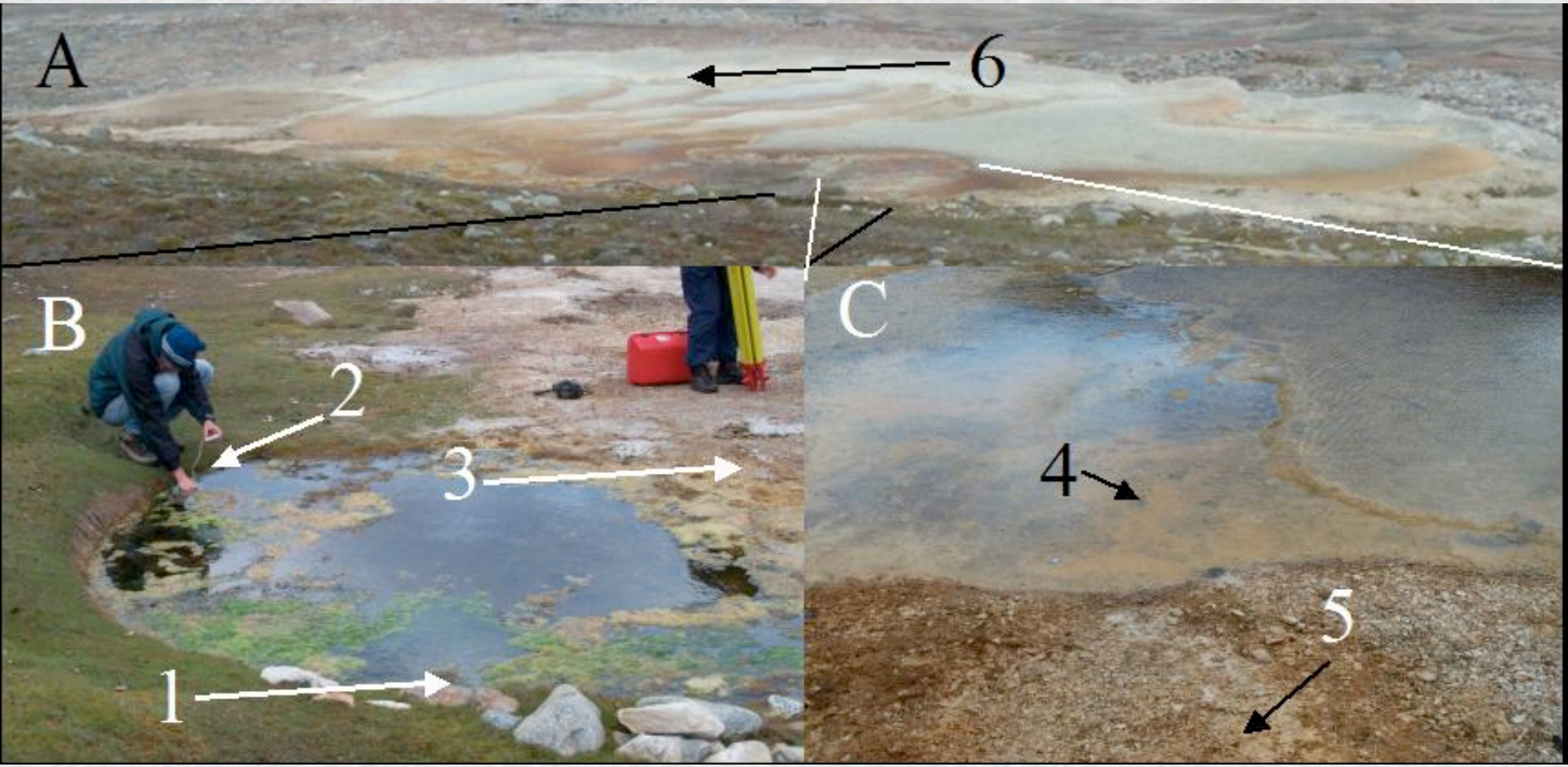
Science Goals

Life detection and characterization in Mars analogue environment

How did the Carbonate globules form – biotic V abiotic

How do the organisms in the warm springs contribute to the formation of the carbonate terraces

Troll Springs

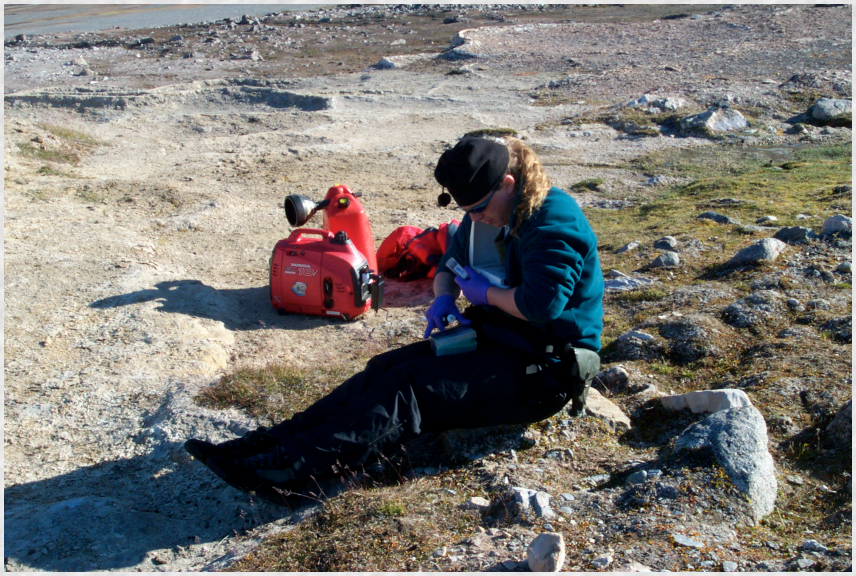


Primer Pair	Target
B27F – 1492R	Generic Eubacteria
PufM.557F – PufM.750R	Specific – purple sulfur bacteria*
GS.619F – GS1144R	Specific – Green sulphur bacteria 16S rDNA
CFX.856F – CFX.1240R	Specific - Green Non sulphur bacteria 16S rDNA
HB.418F – HB.1159R	Specific - Heliobacteria 16SrDNA
NirK.F – NirK.5R	Specific – Nitrate reductase
Amoa.1F – Amoa.2R	Specific – Ammonia Oxidase
APS1.Fb – APS1.Rb	Specific - APS reductase
FDH.F – FDH.R	Specific – Formate Dehydrogenase
McrA.1F – McrA.1R	Specific – Methyl coenzyme M reductase (methanogens)
Nu-SSU-0817 – Nu-SSU-1536	Generic - Eukaryotes

Primer /	Eub	Euk	Pb	GS	GNS	HB	Nr	Ao	APS	FDH	McrA
Area 1	+++	-	+	+	-	++	+	-	++	++	-
2	+++	-	+++	+++	+	-	+	-	+++	+	+++
3	+++	-	+++	-	-	-	-	-	++	+	-
4	+++	-	+++	-	-	-	-	-	-	+	-
5	+++	-	+++	-	-	-	-	-	-	-	-
7	+++	+	+++	-	-	-	-	-	+++	+	-
Blank	+	-	-	-	-	-	-	-	-	-	-
PCR blank	-	-	-	-	-	-	-	-	-	-	-

Eub – Eubacterial; Euk – Eukaryotic; Pb – Purple Bacteria; GS – Green sulphur bacteria; GNS – Green Non sulphur bacteria; HB – Heliobacteria; Nr – Nitrate reductase; Ao – Amoonia oxidase; APS – APS reductase; FDH – Formate dehydrogenase; McrA – Methanogenesis. - - negative; + - weakly positive, ++ - medium positive (similar band fluorescence to ladder) +++ - strongly positive.

In – Field ATP and LAL

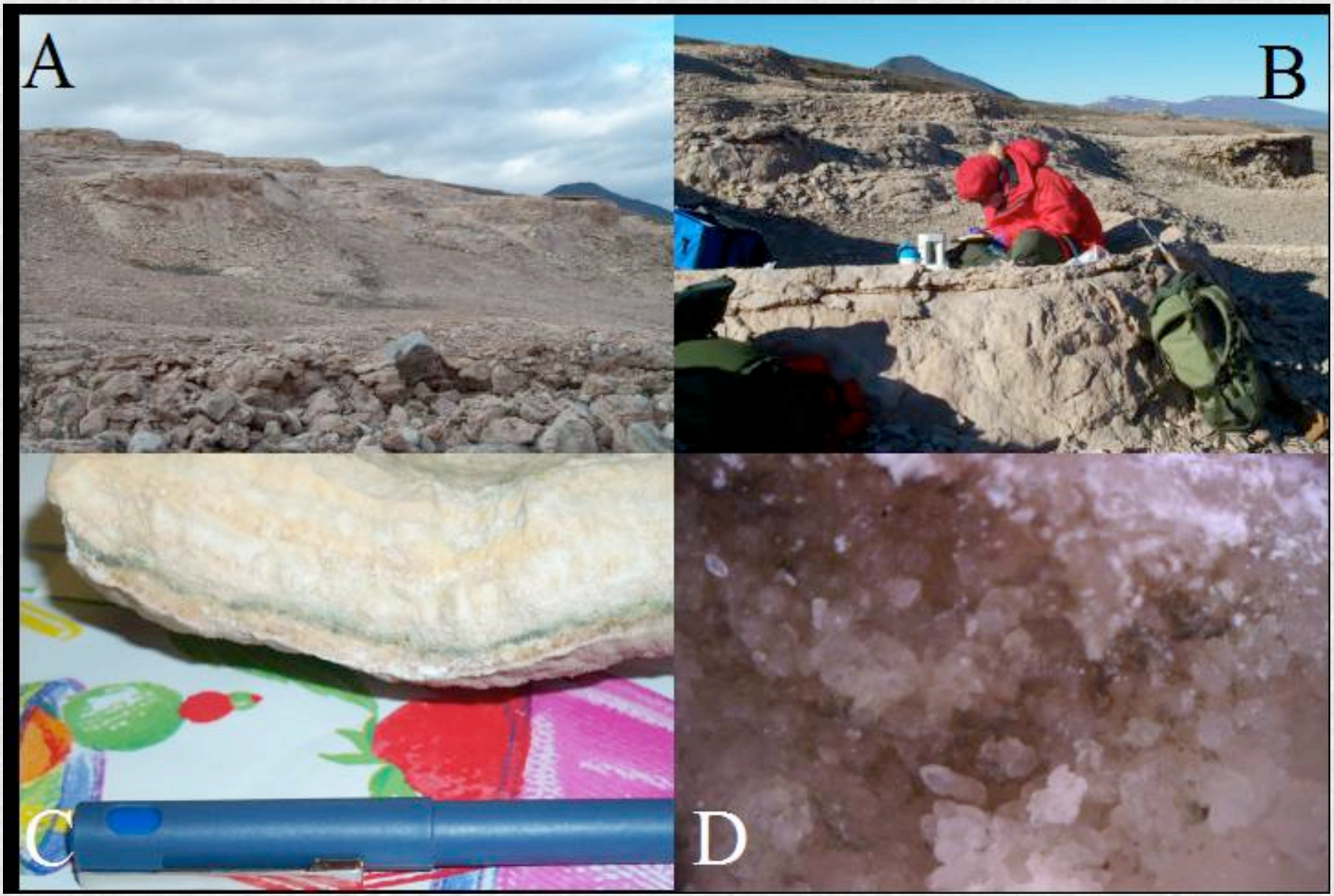


Area	1	2	3	4	5	6	7
ATP	6140	229	5644	2383	NM	1730	NM
LAL	>0.155	<0.100	0.340	0.648	<0.100	NM	0.463

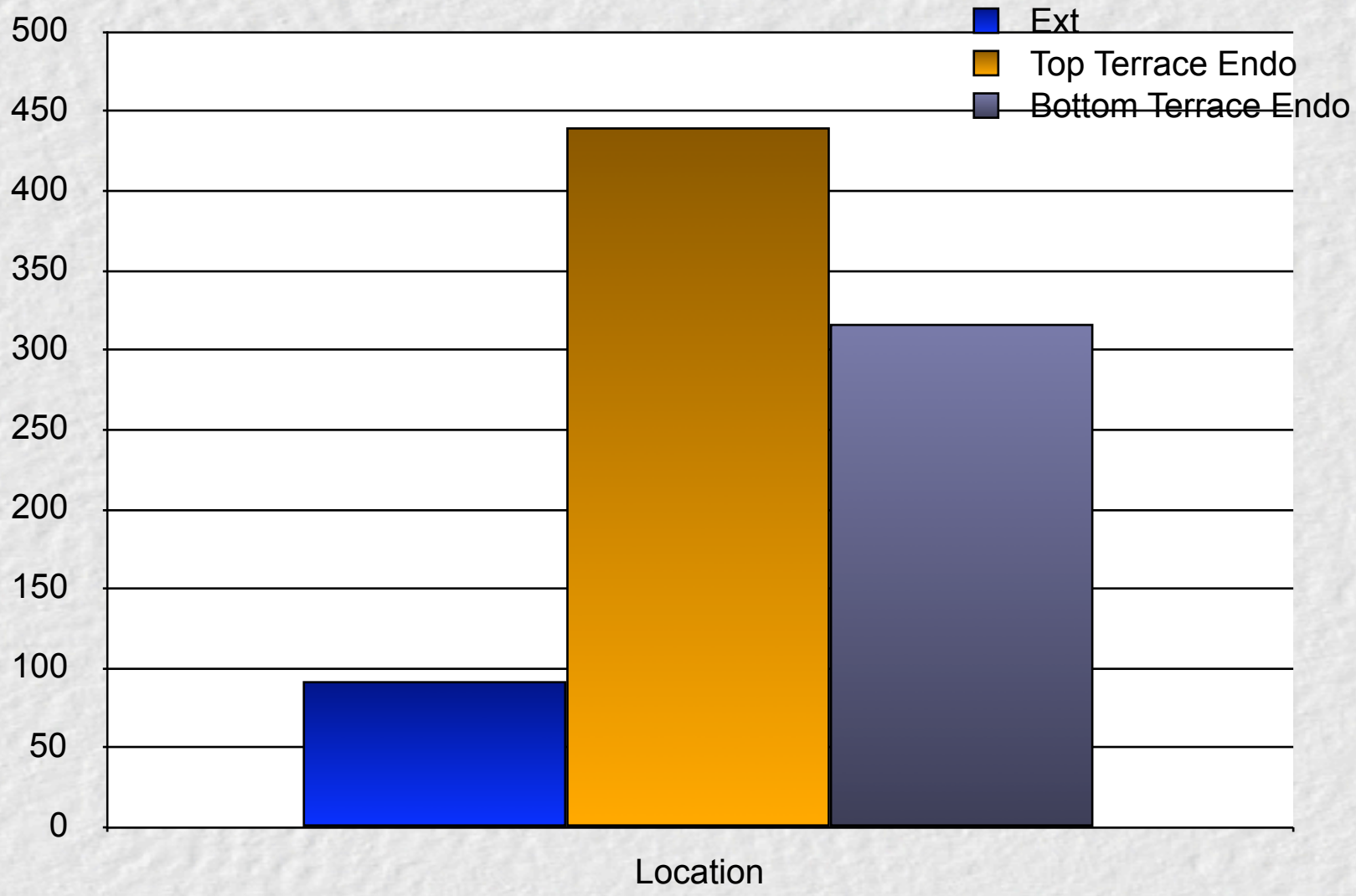
Lab verification of these results (and PCR) is underway. Metabolism and cell mass do correlate in certain areas but do not in others, however all detect life.

Heater on LAL assay needed and has been improved

Carbonate terraces – Troll springs



ATP – Analysis of cryptoendolithic communities

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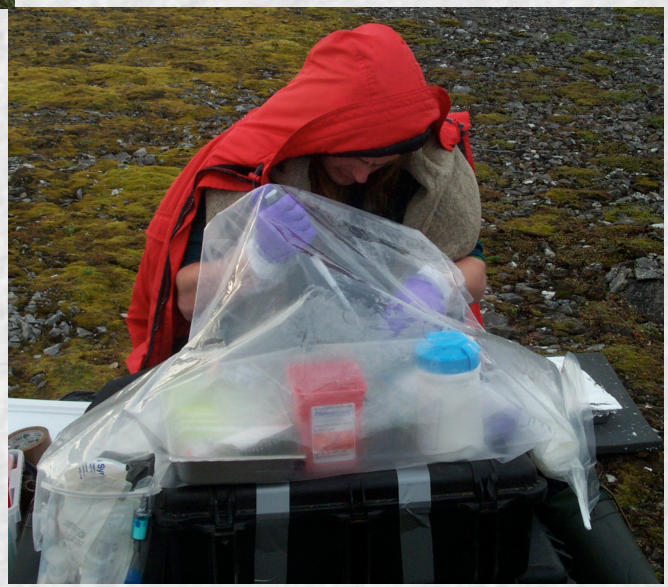
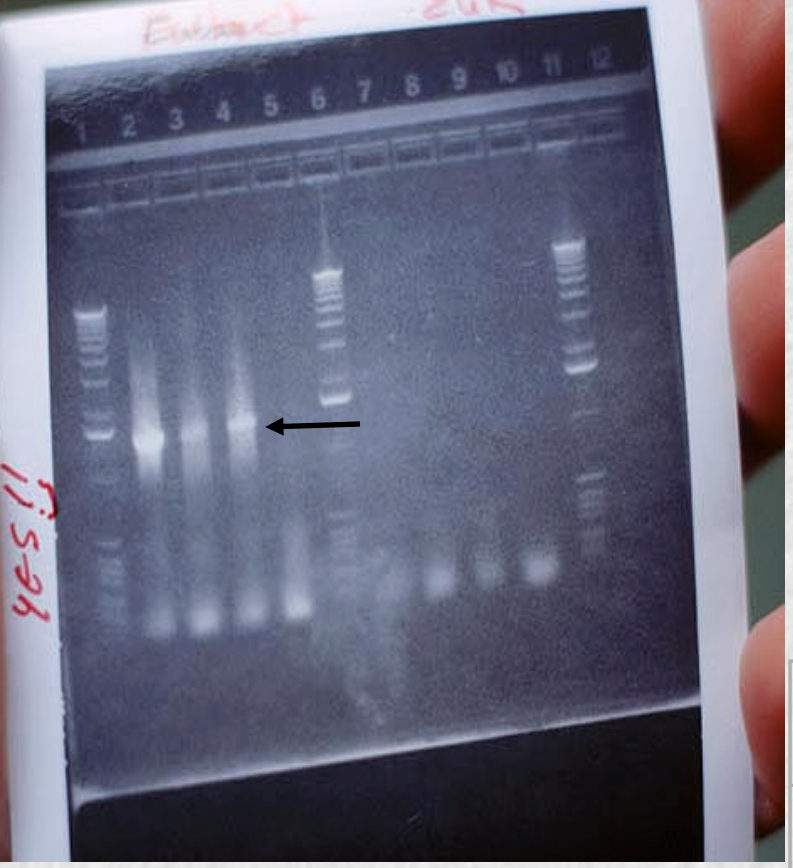
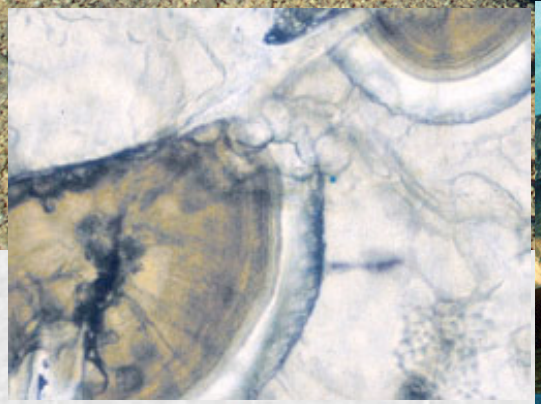


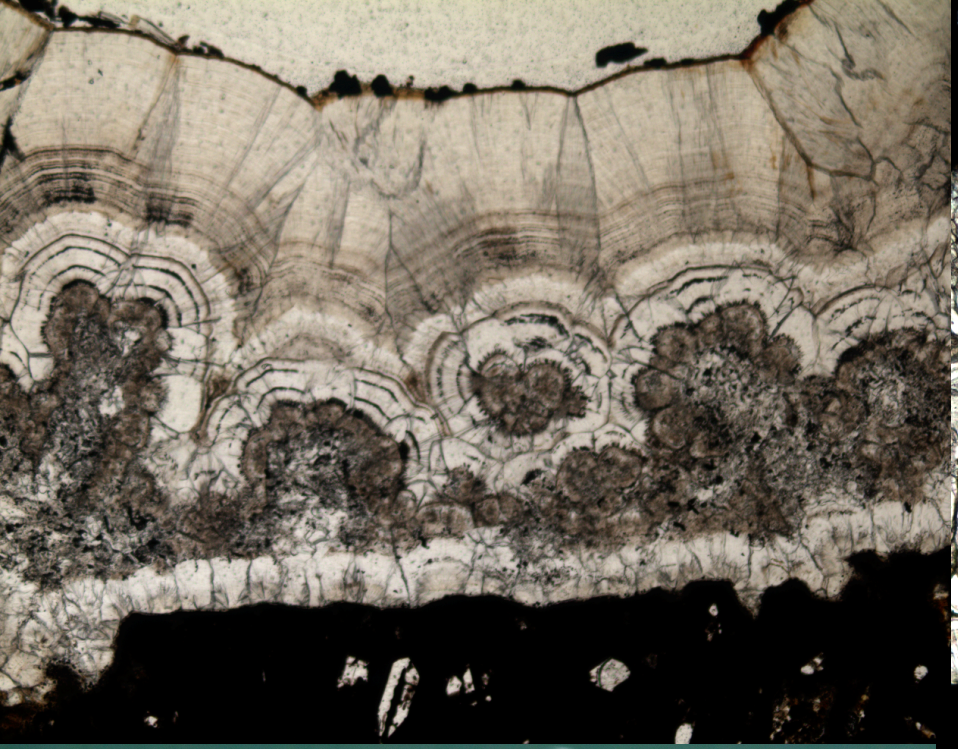


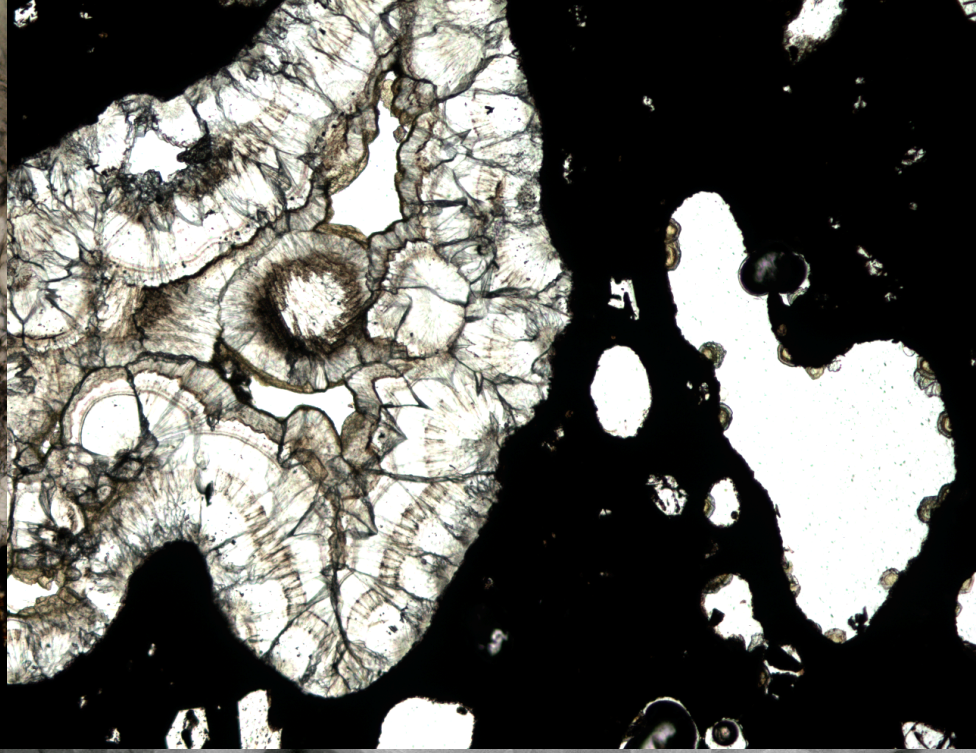
• Sample 1 – Free floating filaments emerging with the spring water.
• Sample 2 – Biofilm filaments along the wall of the spring
• Sample 2b - Biofilm filaments along the wall of the spring
• Sample 3 – Rubbing of the FTA paper along green (cyanobacterial biofilm) on the wall of the cave above the spring.
Blank – Single disk of unused FTA paper that underwent same extraction protocol as the samples outlined above.

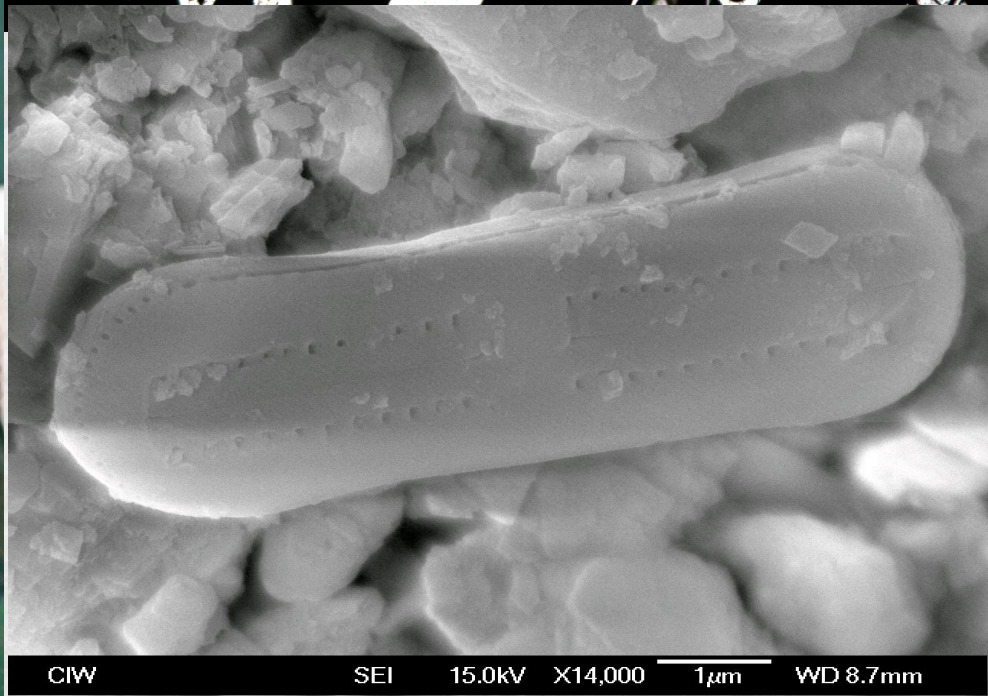
	Eub	Euk	APS	Pb	GS	McrA
1	+++	-	++	-	-	-
2	+++	-	+	-	-	+
2b	+++	-	+	-	-	-
3	-	-	-	-	-	-
Blank	-	-	-	-	-	-

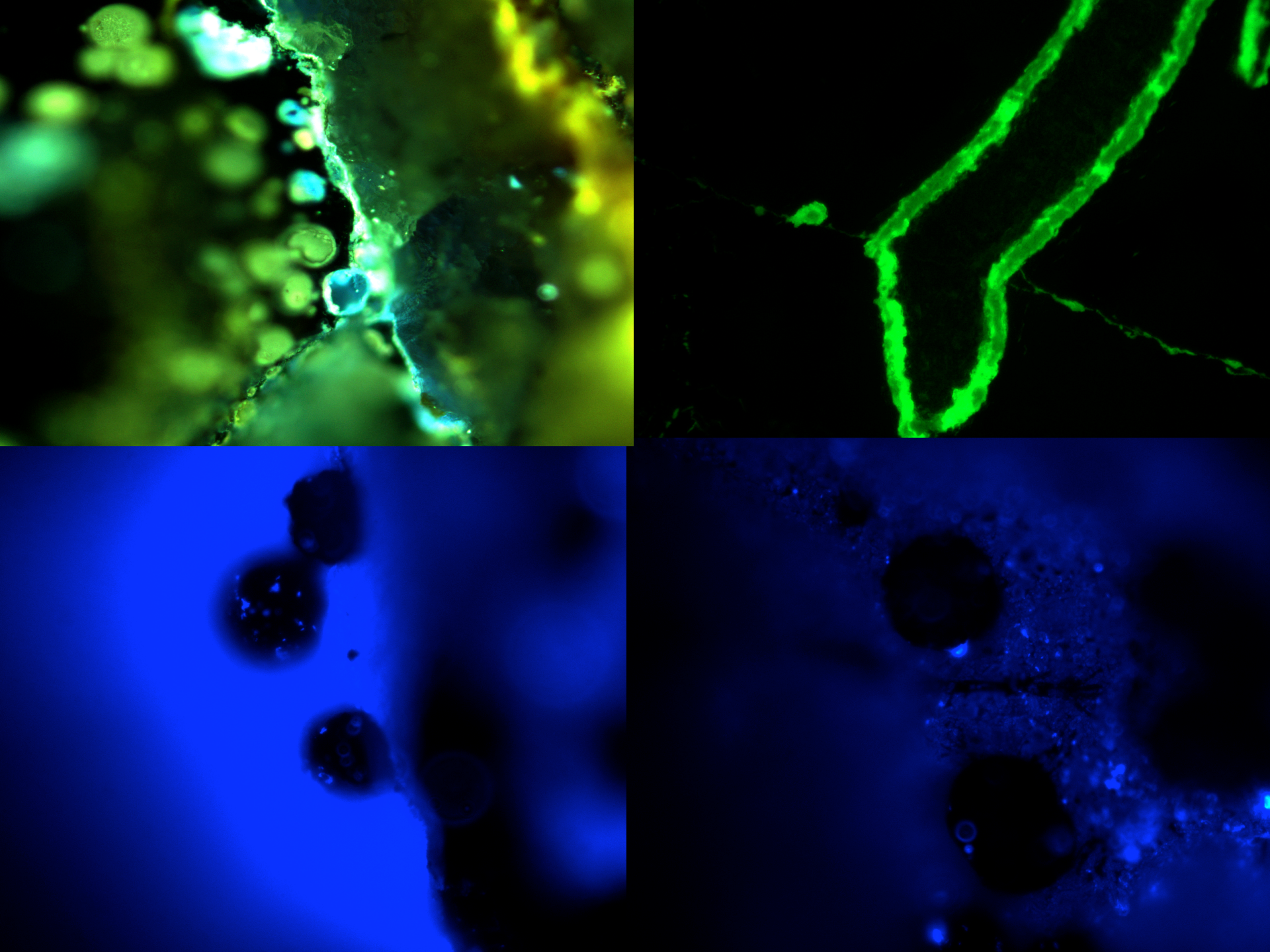
Carbonates

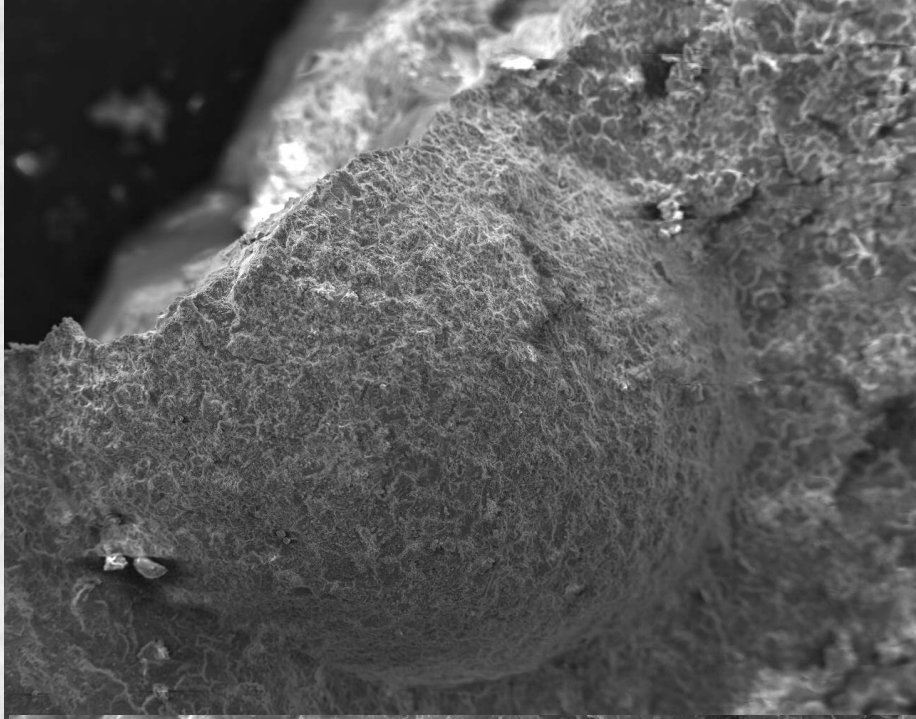


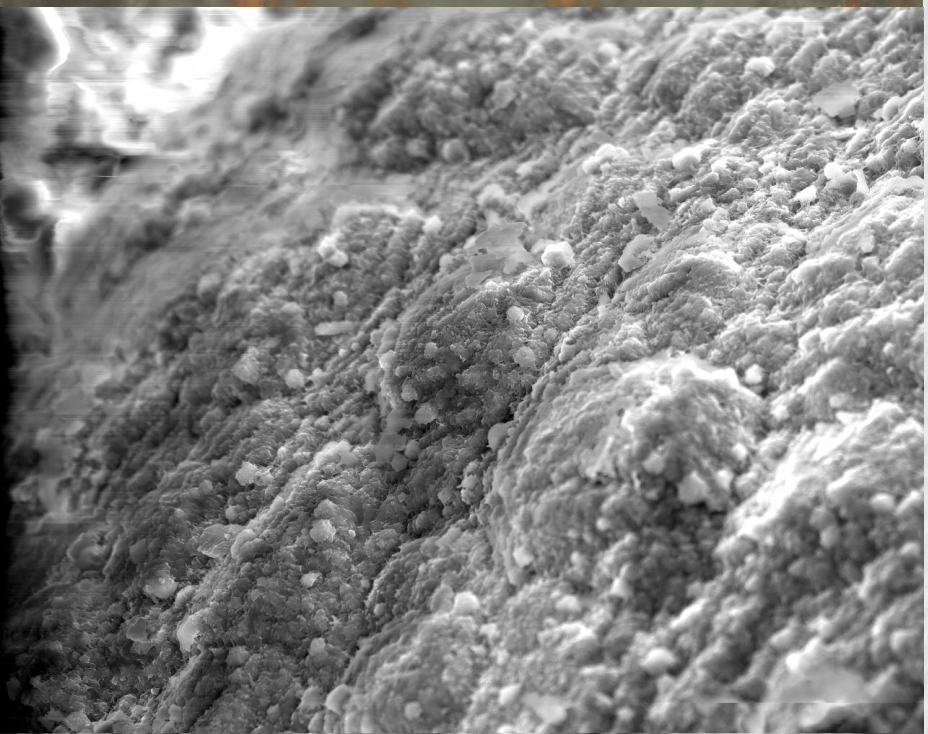


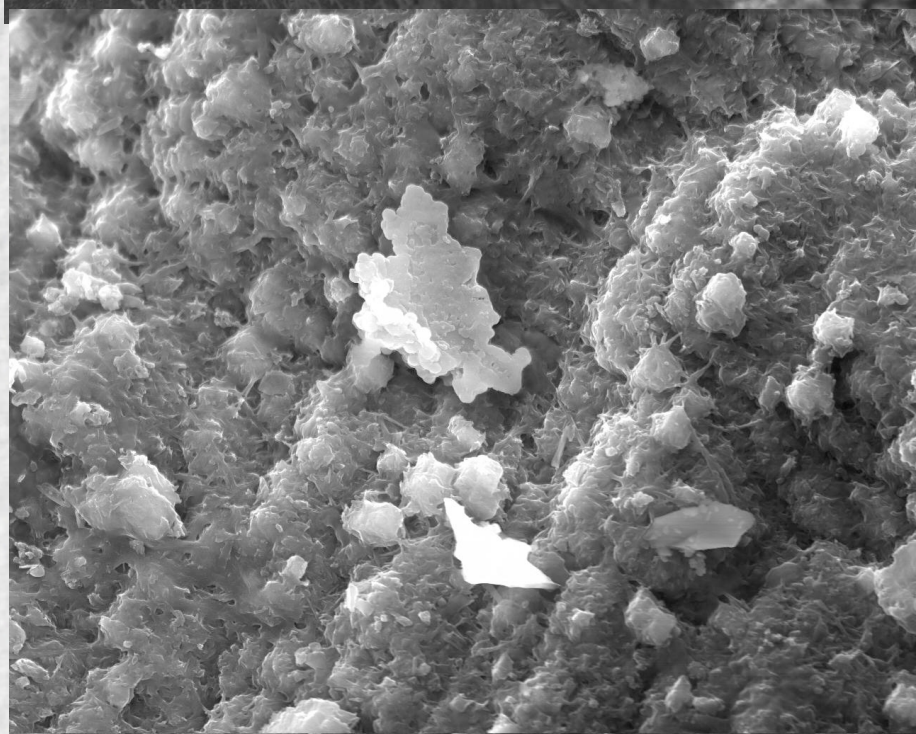



CIW SEI 15.0kV X14,000 1μm WD 8.7mm









ASTEP Conclusions

We have defined an instrument suite that works in labs and in field for the characterization of microbes

Addressed contamination issues

Defined a simple platform for instrument testing

Looking to collaborate with any and all who wish to use such an instrument suite for verification of their instruments or as a stand alone package

Future

Other instruments and deploy array based instruments when possible

Check other life detection strategies

Refine platform design

Svalbard -------

Done 3 environments, several more this year with Pan

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MASSE team

MARTE team